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Herbicidally Active Aminomethylenebisphosphonic Acids

Paweł Kafarski,[1] Barbara Lejczak,[1] and Giuseppe Forlani[2]

[1]*Institute of Organic Chemistry, Biochemistry and Biotechnology, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland*

[2]*Department of Genetics and Microbiology, University of Pavia, via Ferrata 1, I-27100 Pavia, Italy*

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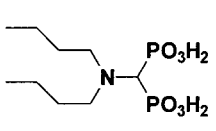
ABSTRACT: *Herbicidal derivatives of aminomethylenebisphosphonic acids discovered over 20 years ago were considered to be some kind of curiosity until 1995. Renewed interest in these compounds brought controversies about their modes and cellular targets of action.* © 2000 John Wiley & Sons, Inc. Heteroatom Chem 11:449–453, 2000

Bisphosphonates have been employed as therapeutic agents for treatment of bone disorders for more than 20 years, and their mode of action still remains unclear [1,2]. Interestingly, the discovery, claimed in the 1979 patent literature [3,4], that these compounds exhibit herbicidal action had received nearly marginal attention up to 1995. Renewed interest in this class of herbicides resulted in several patents and articles dealing with their herbicidal activity and possible molecular targets for their action.

First reports on herbicidal activity considered derivatives of aminomethylenebisphosphonic acids containing aliphatic N-substituents, with the di-*n*-butyl compound **1** being the representative example [2]. This compound when applied at 100 ppm caused over 90% control of studied weeds. Heterocyclic derivatives, especially those containing *N*-pyridyl substituents (with compound **2** being the most effective), showed similar herbicidal activity and were even more effective in field tests [3].



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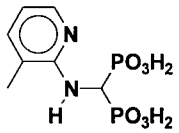

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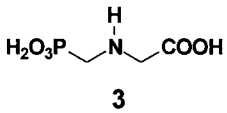

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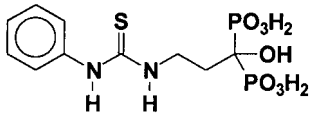
Physiologic activity of *N*-pyridyl-substituted aminomethylenebisphosphonic acids tested against several plant species, as well as plant cell cultures, confirmed their strong herbicidal properties [3–7]. The main phytotoxic indicators observed on whole plants were browning and swelling of the shoots, and strong hypocotyl chlorosis with no effect on their width and branching. Growth is slowed substantially a few days after application, and desiccation of plant tissues becomes pronounced after two weeks. These indicators are similar to those exerted by the popular phosphonate herbicide glyphosate (compound **3**). It is also worthwhile to note that the significant consistency of the effectiveness of *N*-pyridyl-substituted aminomethylenebisphosphonic acids on cell culture and whole plant growth suggests that their toxicity could be exerted through inhibition of some pathways in primary metabolism [7]. Quite interestingly, compounds of high herbicidal activity also rank among the highest active inhibitors of bone resorp-

Correspondence to: Paweł Kafarski.

tion in the Schenk rat model, as well as among inhibitors of *Dictyostelium* amoebae growth [8].

Structure–activity relationship studies did not help very much in understanding the mode of action of these herbicides because there was no reasonable correlation between the structures of the active compounds and their herbicidal effects [9]. First attempts suggested, however, that the presence of two strongly acidic phosphonate residues and one positively charged amino group may be required in order to obtain herbicidal action. Further studies indicated that a positively charged amino group is not an essential structural fragment for activity since compounds, in which this group was replaced by hydroxyl (for example compound **4**) or a urethane moiety (as in representative compound **5**) still remained highly active [10].



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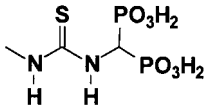

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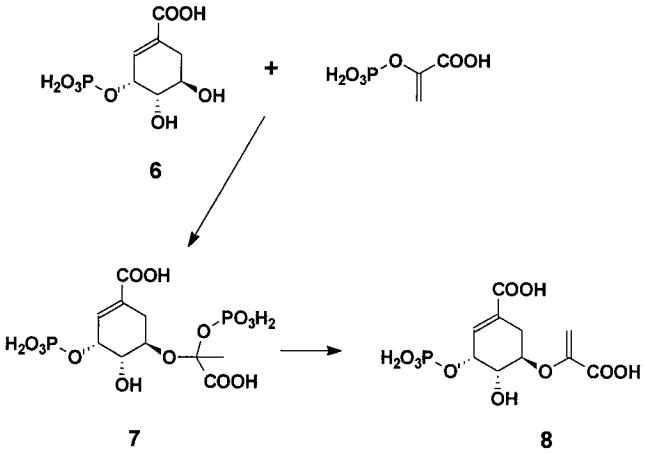
Structural features of *N*-pyridyl-substituted aminomethylenebisphosphonic acids and their resemblance to glyphosate suggested 5-*enol*-pyruvoylshikimate-3-phosphate synthase (target enzyme for glyphosate, that catalyzes the sixth step in the initial common route of aromatic amino acid biosynthesis) as a possible site for the action of these compounds. Moreover, the flat pyridyl ring seemed to be a good mimic of the nearly planar chair conformation of the substrate (shikimate-3-phosphate, **6**), intermediate (**7**), as well as the product (**8**) of the reaction catalyzed by this enzyme (Scheme 1) [11]. This suggestion was additionally supported by the fact that most *N*-pyridylaminomethylenebisphosphonic acids significantly depressed buckweed anthocyanin biosynthesis in vivo [7]. The depression of light-induced anthocyanin biosynthesis is a standard test indicating the influence of a studied compound on the biosynthesis of aromatic amino acids. This appeared, however, not to be the case since the in vitro activity of 5-*enol*-puruvoylshikimate-3-phosphate synthase was found to be unaffected [12].

The first step in aromatic metabolism is the condensation of phospho*enol*pyruvate with erythrose-4-phosphate catalyzed by 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase (Scheme 2). This enzyme has been shown to occur in plants as two distinct isoenzymes with different roles and properties. One form, localized in the cytosol, strictly requires the presence of divalent cations (Co^{2+} or Mg^{2+}), whereas the activity of the other plastidial enzyme does not absolutely require the presence of divalent cations, but is stimulated three- to five-fold by the presence of Mn^{2+}.

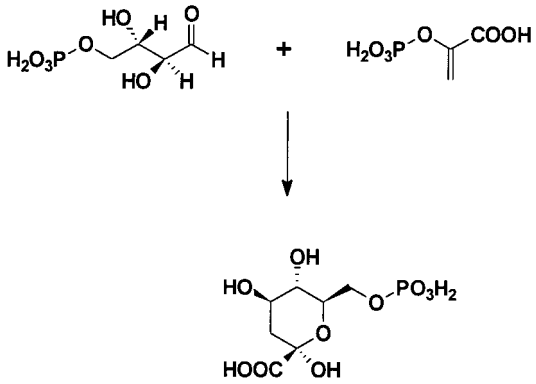
Three out of seven studied biphosphonates (Scheme 3; compounds **13, 14,** and **15**) significantly affected the activity of both isoforms of the enzyme [13].

The activity of the Co^{2+}-dependent enzyme was similarly affected by the presence of all compounds tested, and this effect was completely abolished when the concentration of divalent cations was raised. This clearly showed that inhibition of this enzyme relies upon chelating properties of all the studied compounds. In contrast, only five compounds



6 **7** **8**

SCHEME 1 Reaction catalysed by 5-*enol*-pyruvoylshikimate-3-phosphate synthase.

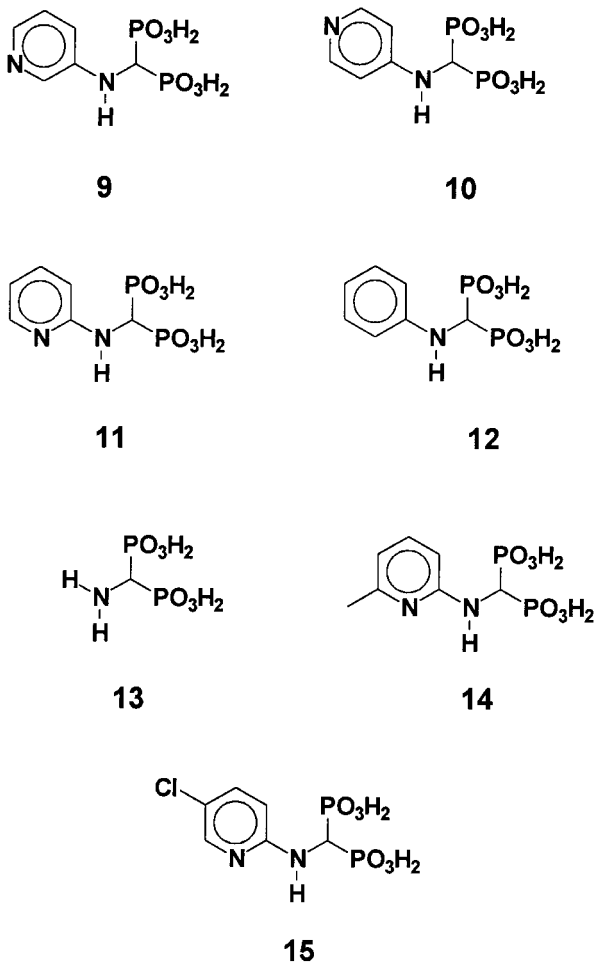


SCHEME 2 Reaction catalysed by 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase.

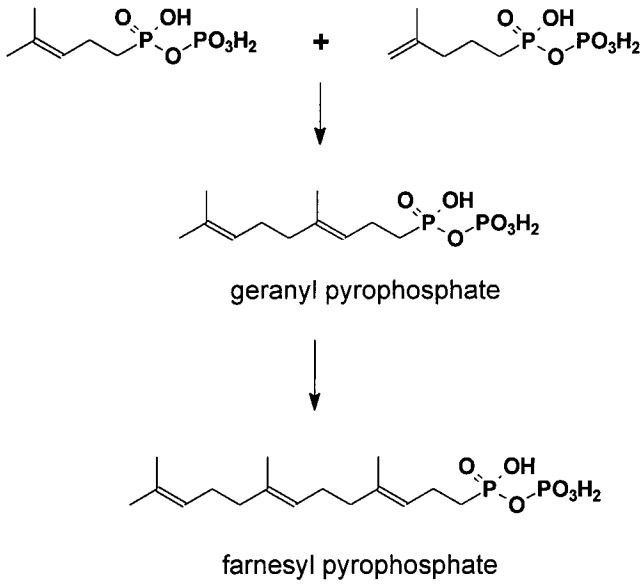
out of seven inhibited Mn^{2+}-dependent isoenzyme, and to a various extent, with the three mentioned previously being the most active. The action of compound **13** was reversed by an elevated level of magnesium ions, and in the case of compounds **14** and **15**, the inhibition was not simply based upon metal chelation. A kinetic analysis indicated that the most effective compound **15** inhibits enzyme competetively with respect to the substrate erythrose-4-phosphate, ruling out the possibility of an inhibition based upon its metal chelating properties. Compound **14** showed noncompetitive inhibition with respect to both substrates (phospho*enol*pyruvate and erythrose-4-phosphate). Amino acid pool measurements of tobacco grown in the presence of this compound pointed out the actual reduction of free aromatic amino acids, showing that the inhibition of this enzyme takes place in vivo, and suggesting that the interference of this aminophosphophonate with plant aromatic biosynthesis may account for a large part of its phytotoxicity [14]. The herbicidal action of compound **15** is somewhat more complicated and will be discussed later.

In contrast to these results, studies on the molecular mode of action of compound **2** did not support the hypothesis that aromatic amino acid biosynthesis may be a target for this bisphosphonate [15,16]. Finding that action of this compound is accompanied by a significant decrease of carotenoid and chlorophyll biosynthesis suggested the isoprenoid pathway as the possible molecular site of the action of this compound. Detailed studies have shown that this compound is a very potent inhibitor of the conversion of isopentenyl pyrophosphate to farnesyl pyrophosphate [6,15,16], a reaction catalyzed by farnesyl pyrophosphate synthase (prenyl trasferase), which is also a metal-dependent enzyme (Scheme 4). Studies carried out at Zeneca laboratories have shown that, among more than 200 structurally diverse bisphosphonates, only those affecting significantly farnesyl pyrophosphate synthase exhibited good herbicidal properties [15]. It is not clear, however, if compounds **13, 14,** and **15** were evaluated in this test.

Farnesyl pyrophosphate synthase is also considered as a molecular target of antiresorptive action of bisphosphonates in bones. Inhibition of this enzyme results in inhibition of protein prenylation, thereby disrupting signaling and ultimately inducing apoptosis [17,18]. Using molecular modeling and ab initio quantum chemical calculation, the possibility



SCHEME 3 Aminomethylenebisphosphonates studied for their inhibitory activity toward 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase.

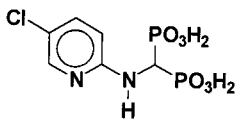


SCHEME 4 Reactions catalyzed by farnesyl pyrophosphate synthase.

that bisphosphonates may act as aza-izoprenoid transition state analogs was shown [19]. The two phosphonate groups of compound **2** (as well as other antiresorptive bisphosphonates studied) readily dock into the diphosphate–Mg^{2+} binding site in farnesylpyrophospate synthase while the charged pyridinium group acts as a carbocation transition state analog, whose binding is stabilized by a cluster of oxygen atoms in the active site cleft, and an overall negative electrostatic potential in this region.

It is generally accepted that the block of carbon flow in the shikimate pathway leading to the biosynthesis of aromatic amino acids decreases phenylalanine and tyrosine levels, while increasing the total amount of free amino acids in plant cell cultures. In order to ascertain that 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase could represent the main target of compound **15** in vivo, studies of the effect of sublethal concentrations of this compound on the pool of free amino acids in cultured *Nicotiana plumaginifolia* cells were undertaken [20]. The results obtained with compound **15** showed a strikingly different pattern than that found for glyphosate. Although an actual reduction of the level of aromatic amino acids was found, the total amino acid content was also significantly reduced in treated cells, mainly due to the decrease in the level of glutamine, asparagine, arginine, glutamic acid, and glycine. This may be due to the additional inhibition of enzymes involved in nitrogen assimilation (glutamate synthase and glutamine synthetase). A similar decrease of the nonaromatic amino acid pool was reported previously for two potent inhibitors of glutamine synthetase, namely methionine sulphoximine and phosphinothricin. The two enzymes were assayed in the presence of a milimolar level of compound **15,** and although glutamate synthase was found to be unaffected, the activity of glutamine synthetase was markedly inhibited. Toward purified enzyme, this compound exerted inhibition of the uncompetitive type with respect to all three substrates. Unlike phosphinithricin, which inactivates glutamine synthetase irreversibly, compound **15** binds reversibly to the enzyme, and this could account in part for the noteworthy effectiveness of the compound in vivo, as compared to that of the former. Thus, inhibition of both aromatic amino acids and glutamine biosynthesis might be an unusual mode of action of herbicidal compound **15** [20].

The chemical structure of compound **15** seems to be completely unrelated to the structures of substrates and products of glutamine synthetase, namely glutamine, glutamic acid (**16**), ADP, or ATP (**17**), and therefore it is difficult to imagine how it is bound by the enzyme. This problem was solved using ligand design methodology proposed by Böhm [21] and implemented in a commercially available program LUDI [22]. Similarly, as in the case of farnesyl pyrophosphate synthase, the two phosphonate moieties readily dock into the binding site of the enzyme containing two Mn^{2+} ions. Flat, aromatic, and rich in electrons, the pyridine ring is placed in a cleft composed of glutamic acid and arginine residues, a site of unknown physiological importance (P. Kafarski, unpublished results, 2000).



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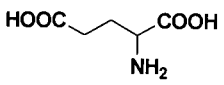

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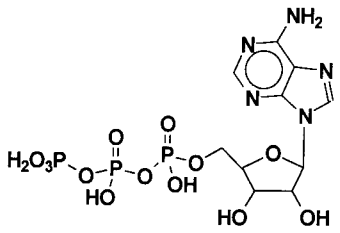

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Bisphosphonates also appear to be in the class of effective, competitive inhibitors of pyrophosphatase from mung bean vaculoe [23]. This enzyme is believed to be responsible for maintaining the vacuolar proton gradient. Pyrophosphatase is also a metal-dependent enzyme and contains Mg^{2+} in its catalytic site. A complex of pyrophosphate with magnesium is a real substrate of the enzyme. In this case, bisphosphonates are considered as nonhydrolyzable analogs of pyrophospate, and the enzyme is preferentially inhibited by simple aliphatic compounds, although the presence of an amino group in their structure improved inhibitory properties in some cases. Compound **9** appeared to be a moderate inhibitor of this enzyme. Bisphosphonates also showed similar activity against mitochondrial H^+-pyrophosphatase [24].

It is also worthwhile to note that some bisphosphonates strongly inhibit mammalian squalene synthase [25], but this site of action has not been considered as a possible target for herbicidal action so far.

CONCLUSIONS

Derivatives of aminomethylenebisphosphonic acids constitute a new class of promising herbicides. Their

targets at cellular level are still poorly understood, but the available biochemical data suggest that they should be considered as a heterogenous group of compounds with various modes of action. It is most likely that their herbicidal activity derives from their simultaneous action on several enzymes.

N-pyridyl-substituted aminomethylenebisphosphonic acids, the mode of action of which was the most extensively studied, are quite simple organic molecules. However, their metal complexing abilities vary in a manner strongly dependent on the kind and position of substituents introduced into the pyridyl ring (E. Matczak-Jon, unpublished results, 2000). Since all the reported enzymes inhibited by bisphosphonates are metal-dependent ones, the complexing properties of these compounds may be of vital importance for the exerted inhibition and require more detailed studies.

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